Journal of Chromatography, 427 (1988) 151–156
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 4109

Note

Rapid confirmation of enzyme-multiplied immunoassay test (EMIT) for phencyclidine-positive urine samples with capillary gas chromatography–nitrogen–phosphorus detection

KARL VEREBEY*,* and ANN DePACE

New York State Division of Substance Abuse Services, Testing and Research Laboratory, 80 Hanson Place, Brooklyn, NY 11217 (U.S.A.)

(First received July 30th, 1987; revised manuscript received January 4th, 1988)

Phencyclidine (PCP) was developed in the 1950s as an intravenous anaesthetic agent with minimal cardiac and respiratory depressant effects. PCP was promising but post-operative psychotic reactions in some patients forced its withdrawal from medical use. Currently PCP is part of the illegal drug armamentarium. Its dangerous effects on behavior and driving requires monitoring when illegal drug abuse is suspected.

Drug abuse fads change with time depending on availability and attitudes toward particular substances of abuse. Currently, in the U.S.A. marijuana and cocaine are much more prevalent than PCP, yet PCP remains a commonly abused substance [1]. Its persistence in street drugs is probably due to cheap and easy PCP synthesis. PCP is often mixed into cocaine and marijuana preparations or substituted for other street drugs and sold to unsuspecting buyers [2]. In many toxicology laboratories enzyme-multiplied immunoassay test (EMIT) is used for PCP identification in urine [3]. PCP-positive urine samples must be confirmed by scientifically alternative techniques such as gas chromatography (GC) or gas chromatography–mass spectrometry (GC–MS). This is necessary to ensure the accuracy and specificity of the EMIT screening technique [3,4]. Several GC methods are described in the literature for PCP identification and quantitation [5–8]. The numerous preparatory steps and derivatization render these methods too slow for routine confirmation of large numbers of samples. This communi-

*Academic affiliation: Department of Psychiatry, SUNY Health Science Center at Brooklyn, Brooklyn, NY 11203, U.S.A.

0378-4347/88/$03.50 © 1988 Elsevier Science Publishers B.V.

cation describes a capillary GC method with a reference standard of caramiphen edisylate, for the rapid confirmation of PCP-positive urine samples by EMIT.

EXPERIMENTAL

Materials

The internal or reference standard, caramiphen edisylate, was purchased from Sigma. PCP was a gift from Dr. Richard Hawks, National Institute on Drug Abuse; *n*-butyl chloride was Resi-Analyzed®, purchased from J.T. Baker and ethyl acetate was glass-distilled, purchased from Burdick and Jackson Labs. Sodium hydroxide, 50% solution, was purchased from Fisher Scientific.

Extraction

To siliconized conical centrifuge tubes, 0.2 ml of urine, 0.8 ml of deionized water and 50 μl of 50% sodium hydroxide were added. Caramiphen (100 ng), the internal standard, was also added and the solutions were mixed by vortexing. *n*-Butyl chloride (5 ml) was added as the extracting solvent. After shaking on a mechanical shaker for 10 min and centrifugation for 5 min at 750 *g* the upper layer (*n*-butyl chloride) was transferred to clean siliconized 15-ml centrifuge tubes, leaving about an 0.5-ml portion of solvent behind. The organic solvent was then evaporated to dryness under a gentle stream of nitrogen at 50°C. The residue was reconstituted with 25 μl of ethyl acetate and 1–2 μl were injected into the gas chromatograph.

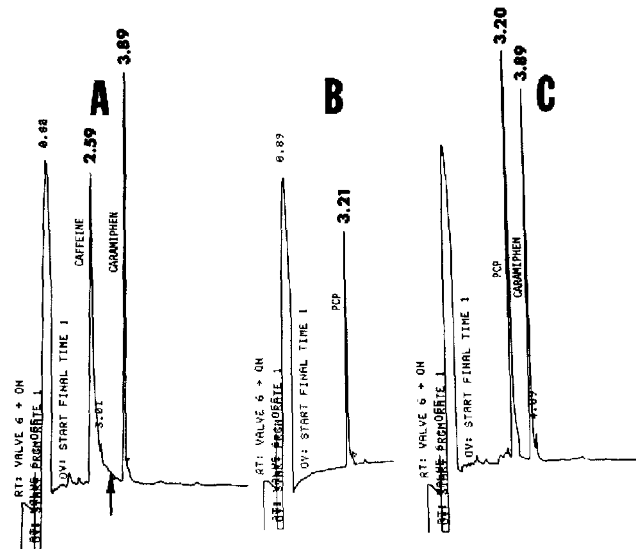

Fig. 1. (A) Blank urine sample containing caffeine with the reference standard, caramiphen, added. The arrow shows the PCP zone. (B) PCP standard. (C) Tracing of an EMIT PCP-positive urine sample; the reference standard, caramiphen, was added.

Elimination of the caffeine peak

After addition of 0.8 ml water and 50 μl of 50% sodium hydroxide to 0.2 ml of urine samples, the tubes were heated at 75°C for 15 min. Following extraction and chromatography, the caffeine peak disappeared from the chromatographic tracings while the PCP peak was unaffected (see Fig. 2B).

Instrumentation

A Hewlett-Packard 5880 gas chromatograph was used with capillary column capability and equipped with an alkaline flame ionization detector. The column was a 15 m$\times$0.2 mm I.D. WCOT fused-silica capillary column with 0.25-μm DB-1 coating. It was purchased from J. and W. Scientific. The instrument was operated in the splitless mode. The injection port and detector temperatures were 190 and 300°C, respectively. The column temperature was programmed from 160 to 190°C at a rate of 30°C/min. Flow-rates were: helium (carrier gas), 1.0 ml/min; hydrogen, 3 ml/min; air, 90 ml/min. The retention time for PCP was 3.20 min and that of caramiphen 3.89 min (Fig. 1).

Sensitivity and recovery

The EMIT PCP assay has a cutt-off between positive and negative samples at 75 ng/ml PCP concentration [3]. Therefore the GC method has to be at least as sensitive as the EMIT in order to be useful as a confirming technique. To each four 0.1-ml blank urine samples, 7.0 ng PCP were added, extracted according to the method and 3.0 μl of the 25 μl ethyl acetate were injected into the chromatograph. The resulting PCP peak representing 0.84 ng of extracted PCP produced a 30$\pm$3 mm response on the chromatographic tracings. About one third of this value, 300 pg PCP, is the minimum detectable analyte after extraction. This value is very close to the absolute sensitivity of the instrument for PCP since the recovery as shown below is close to 100%. The baseline was clear thus no signal-to-noise ratio can be calculated. Fig. 1A is a tracing of a blank sample with caramiphen added and Fig. 1B shows a tracing of PCP standard. Fig. 1C shows the chromatographic tracing of an EMIT PCP-positive urine sample which does not contain caffeine.

PCP recovery was 98% with *n*-heptane–isoamyl alcohol (98.5:1.5) [5]. In this study *n*-butyl chloride was used as the extracting solvent. Eight 10-ng absolute PCP standards were prepared. The resulting PCP peaks were averaged and compared to peaks obtained by the extraction of 10.0 ng PCP from blank urine according to the method. The calculated recovery of PCP was 96.7$\pm$3.1% (n=8). Other solvents also yielded excellent PCP recoveries, but *n*-butyl chloride extracts were cleaner.

RESULTS AND DISCUSSION

Busy toxicology laboratories using the EMIT PCP assay for drug abuse screening are in need of quick confirmation methods. This GC procedure since its de-

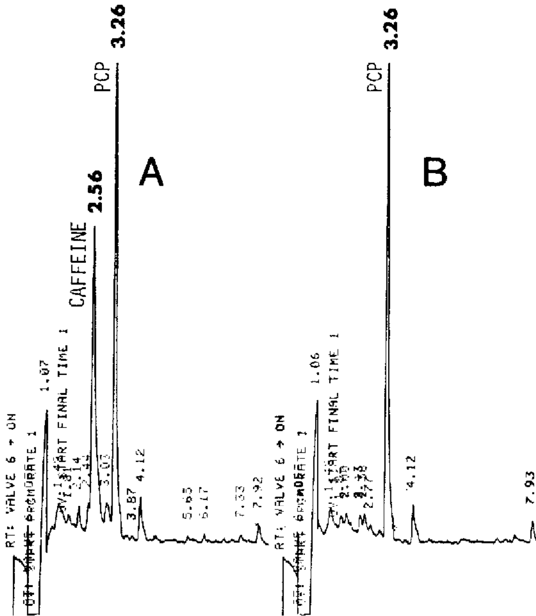

Fig. 2. (A) Both caffeine and PCP are present in the tracing of a PCP-positive urine sample. (B) Aliquot of the same sample is heated at 75°C for 15 min before extraction; the caffeine peak disappeared but the PCP peak was unaffected.

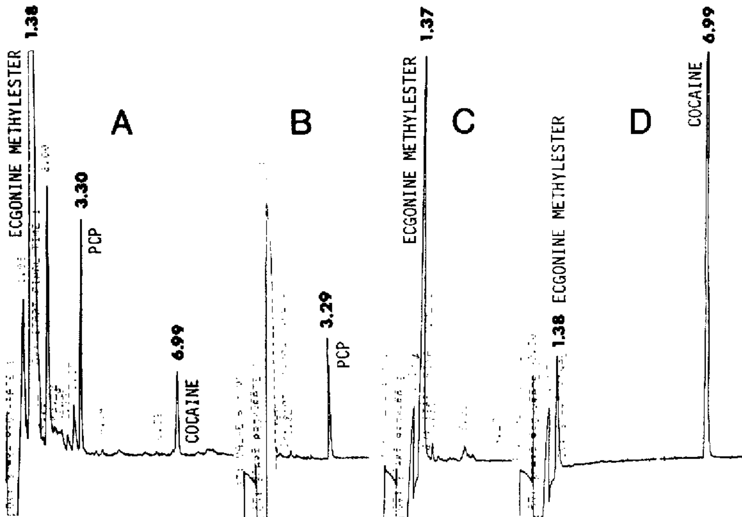

Fig. 3. (A) Tracing of a sample positive for PCP and cocaine by the EMIT PCP and cocaine assays, respectively; ecgonine methyl ester, a major cocaine metabolite (1.38 min), PCP (3.30 min) and cocaine (6.99 min) are present. (B) PCP standard. (C) Ecgonine methyl ester standard. (D) Cocaine standard, ecgonine methylester is also present as a contaminant.

velopment has been used for confirmation of 104 PCP-positive urine samples. Out of the 104 samples 96 samples or 92% were confirmed positive for PCP. Those which could not be confirmed may have had trace amounts of PCP present,

too little to call positive. The EMIT PCP assay cross-reacts with PCP metabolites. Some of the PCP-positive urine samples may be low in PCP and high in PCP metabolite concentration. The capillary GC method detects PCP exclusively and not the metabolites. This may be the reason for the less than 100% confirmation by this method. The internal standard, caramiphen, was chosen because (1) it has a favorable retention time in relation to PCP and good chromatographic properties; (2) caramiphen edilysate is readily available from commercial sources at low prices and large quantities; and (3) caramiphen is an excellent internal standard for the quantitation of PCP.

Just before PCP a peak with the retention time of 2.56 min was identified as caffeine by a pure standard. The caffeine and PCP peaks had baseline separation, thus routinely no interference was observed. On one occasion an extremely large caffeine peak did overwhelm a small PCP peak and selective elimination of the caffeine peak was desirable. An earlier observation prompted us to utilize an alkaline pre-incubation at 75°C before sample extraction which eliminated the caffeine peak (Fig. 2A and B). The procedure described in Experimental did eliminate the caffeine peak, thus the small PCP peak was better visualized. This procedure was seldom needed. It is reported here because it is an interesting observation and for its use if needed.

Often in PCP-positive urine samples ecgonine methyl ester, a major cocaine metabolite, is also present. The ecgonine methyl ester peak emerges before PCP at 1.38 min (Fig. 3A). Cocaine was also present in this sample at 6.99 min. The presence of cocaine in urine indicates that the sample was collected close to cocaine use. This is presumed, because unchanged cocaine is not readily excreted into urine, unless the urine is collected shortly after cocaine administration. Thus, the method used for PCP confirmation is also useful for identification of cocaine and ecgonine methyl ester. Ecgonine methyl ester is excreted in higher concentration than benzoylecgonine (another major metabolite of cocaine) shortly after cocaine use, however, in later samples benzoylecgonine is in higher concentrations [9]. Benzoylecgonine is not detected by this method. Seeing both PCP and cocaine in a sample is of practical value. Many street cocaine samples are laced with PCP, thus the combined use of PCP and cocaine can be identified.

In conclusion, the method described in this paper is practical, quick and sensitive for the confirmation of EMIT PCP-positive urine samples. The limit of detection is 5–10 ng of extracted PCP. Validation of the method resulted in 92% confirmation of EMIT PCP-positive urine samples.

ACKNOWLEDGEMENTS

The authors wish to thank Mildred Bowens for the preparation of the manuscript and Mr. Jed Shaw for the illustrations.

REFERENCES

1 D.A. Gorelick, J.N. Wilkins and C. Wong, in D.H. Clouet (Editor), Phencyclidine: An Update, NIDA Research Monograph No. 64, DHEW, Public Health Service, ADAMHA, Rockville, MD, 1986, pp. 218–228.

2 S.E. Lerner and R.S. Burns, in R.C. Peterson and R.C. Stillman (Editors), Phencyclidine Abuse: An Appraisal, NIDA Research Monograph No. 21, DHEW, Public Health Service, ADAMHA, Rockville, MD, 1978, pp. 66–118.
3 EMIT-dau™ Phencyclidine Assay, SYVA, Palo Alto, CA, June, 1984.
4 K. Verebey, M.S. Gold and S.J. Mule, Psychiatric Ann., 16 (1986) 235–241.
5 E.H. Holsztynskaya and E.F. Domino, J. Anal. Toxicol., 10 (1986) 107–115.
6 G.M. Simpson, A.M. Khajawall, E. Alatorre and F.R. Staples, Clin. Toxicol., 19 (1982–83) 1051–1059.
7 J.R. Woodworth, M. Mayersohn and S.M. Owens, Analy. Toxicol., 8 (1984) 2–6.
8 R.C. Kammerer, E. Di Stefano and D. Schmitz, J. Anal. Toxicol., 4 (1980) 293–298.
9 J. Ambre, M. Fischman and T.I. Ruo, J. Anal. Toxicol., 8 (1984) 23–25.